Vascular effects of continuous hyperbaric oxygen exposure: experimental outlook

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Abstract

Hyperbaric oxygen (HBO) treatment aims to restore tissue oxygenation by inhaling 100% oxygen in pressure rooms. Although beneficial effects have been reported with regard to re-oxygenated ischemic tissues, conflicting findings have been presented concerning the paradoxical tissue response following reperfusion and/or the different responses of non-ischemic normal tissues to increased oxygen exposure. The present study sought to experimentally investigate the impact of continuous HBO treatments on normal aortic tissue. New Zealand rabbits were placed in pressure rooms for 90 minutes per day under 2.5 atmospheric pressure and exposed to HBO for 28 days. Normal structural histology was obtained in the control group. Foam cells were detected in the aortic intimae, thickening and undulation were visualized in the endothelium, and localized separations were observed in the tunica media in the study group compared with the control group. Moreover, salient vasa vasorum was detected in the study group via histopathology. These findings suggest that continuous HBO exposures disrupt the normal vascular structure of a healthy aorta.

Key words: aorta; healthy tissue; histopathology; hyperbaric oxygen; increased oxygen exposure; periodical treatment; vascular damage

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Introduction

Hyperbaric oxygen (HBO) therapy is a specialized adjuvant treatment involving the periodic administration of 100% oxygen in pressure rooms. It has proved particularly effective as a treatment for poorly oxygenated ischemic tissue pathologies such as wounds, and it aims to ensure a higher oxygen supply in cases of hyperoxia-enhanced antimicrobial activity.[1,2] The beneficial effects of HBO treatment in terms of infection-related diseases have been reported in several prior studies. For instance, Kutlay et al.[3] reported postoperative discitis patients to tolerate HBO well when undergoing periodic antibiotic treatment, noting that the treatment modality allowed for effective infection control. Moreover, Escobar et al.[4] reported lower mortality and morbidity rates among necrotizing fasciitis patients who received adjunctive HBO treatment when compared with those who received more aggressive treatments.

However, certain negative effects of systemic exposure to higher O_2 concentrations, as opposed to localized treatment, have also been reported. For example, higher oxygen concentrations induce the formation of reactive oxygen species, while the induced oxidative stress has the potential to exert toxic effects on organisms via cellular reactions.[5,6] In addition, HBO treatments have vascular effects. The induced reactive oxygen species contribute to the production of peroxynitrite ($ONOO^-$) via the reaction with superoxide that inhibits nitric-oxide-mediated vasodilatation, thereby leading to increased vascular resistance as a result of the vasoconstrictive response.[7,8] Unfortunately, the vascular effects of systemic HBO treatment on healthy systems remain unknown.

The present study sought to experimentally investigate the vascular effects of periodical systemic HBO exposure on the healthy animal aorta. Furthermore, it aimed to reveal the differences between the vascular structure of normal aortas and that of aortas exposed to HBO treatment.

Materials and Methods

Ethical approval

After being designated an animal study according to the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, and following the determination of the study steps, ethical approval to conduct the research was obtained from the local animal ethical committee of Dicle University's School of Medicine (approval No. 2015:11:9) on February 18, 2015. All experiments were designed and reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.[9]

Animals

Fourteen male New Zealand white rabbits aged 5–6 weeks (body mass: 2.1–3.3 kg) were obtained from the animal production unit of Dicle University's animal laboratory. Prior to starting the experiment and immediately after the application of HBO, the rabbits were provided with a standard diet and tap water ad libitum, and they were kept in cages programmed with standard humidity (50 ± 5%) and temperature (22 ± 2°C) and subject to a 12-hour light/dark cycle.

Group creation

The 14 rabbits were randomly divided into two equal groups as

follows. In the control group, the rabbits ($n = 7$) were subject to standard cage life (as set out above) without any treatment application. In the study group, the rabbits ($n = 7$) were exposed to HBO treatment (100% oxygen at 2.5 atmospheric pressure [253.3125 kPa]), which was applied for 90 minutes per day in specialized HBO rooms. The study group rabbits were subject to standard cage life (as set out above) after the HBO application. The HBO treatment was applied periodically for 28 days.

Study protocol

All the rabbits were sacrificed via the intravenous administration of a high dose of pentothal sodium (60 mg/mL at a dose of 5 mL/2 kg via ear vein; Abbot Laboratories, Chicago, IL, USA) on day 28. Afterwards, their thoracic and abdominal aortic tissues were obtained for histological examination.

Histopathological evaluation

The obtained aortic tissues were fixed in 10% formalin and then embedded into paraffin blocks for storage prior to histological staining. Later, 6 µm transverse slices were obtained from the paraffin blocks. Hematoxylin-osin staining was performed as follows. The sections were hydrated using an aldehyde-based fixative. Each slide was rinsed for 30 seconds with distilled water. Thereafter, the slide was placed into a Coplin pool containing hematoxylin and rinsed during 30 seconds. The slide was washed again with distilled water. Next, the slide was stained with 1% eosin Y solution for 10–30 seconds while being shaken. Finally, the slide was dried with 95% alcohol.[10] The Masson's trichrome staining was performed as follows. Each slide was treated with a 5% potassium dichromate and 5% trichloroacetic acid solution for 30 minutes and then rinsed in distilled water. Next, it was washed for 5 minutes in a solution containing 0.5% hematoxylin, 1% ferric chloride, 50% ethanol, and 0.1% hydrochloric acid. The subsequent step involved washing the slide with a 2.5% phosphomolybdic acid and 2.5% phosphotungstic acid solution. The slide was then placed with a 1% orange G and 0.25% aniline blue solution for 5 minutes before being rinsed with a 1% acetate solution. After these steps had been repeated several times, each slide was dehydrated in ethanol and xylene and then coverslipped in mounting medium.[11,12]

Finally, the periodic acid-Schiff (PAS) staining was performed as follows. Each slide was prepared, dried, and fixed with aldehyde prior to being washed in 70% ethanol. The slide was treated with a periodate solution for 5 minutes and then washed again with alcohol. Next, the slide was treated with a reducing solution for 5 minutes before being washed in alcohol (70%) once more. The slide was stained with fuchsin-sulfite for 15–45 minutes. Subsequently, it was washed 2–3 times with sulfide washing solution and then stained with aqueous malachite green (0.002%). As the last step, each slide was washed with distilled water, dried, and examined.[12,13]

All the slides were microscopically evaluated using an Axio Imager.Z2 equipped with ApoTome.2 (Carl Zeiss, Göttingen, Germany). The histological evaluations were performed by a laboratory technician who was blinded to the study and control groups.

Results

Normal morphology

Normal histopathological findings were detected in the control group on the basis of the hematoxylin-eosin staining, Masson's trichrome staining, and PAS staining (**Figure 1**). The normal histological morphology of the control group rabbits' vascular lumen and vascular layers (tunica intimae, tunica media, and tunica adventitia) was visualized.

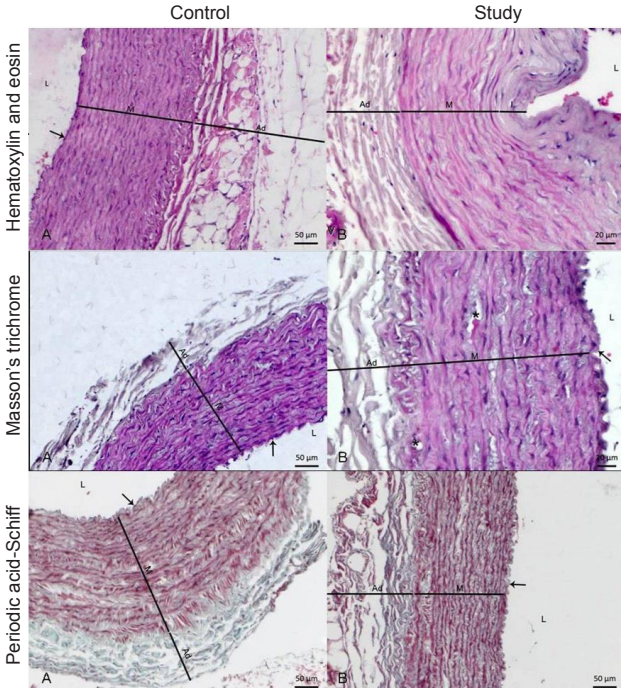

Figure 1: The histopathological comparison of the aorta between normal and hyperbaric oxygen exposed rabbits.
Note: The normal histological structure of aorta in control group. The disrupted aortic structure with medial separations, thickening and foam cells were detected on tunica intimae, prominent vasa vasorums in study (hyperbaric oxygen exposed) group. Black arrow: Tunica intimae; *: separation in media. Ad: Tunica adventitia; I: tunica intimae; L: lumen; M: tunica media; V: prominent vasovasorum. Scale bars: 20 µm in study group with Masson's trichrome and hematoxylin-eosin staining, 50 µm in others.

Moreover, regularly ordered endothelial cells were seen on their intima. The normal distribution of the elastic fibers (large numbers), smooth muscle, and collagenous tissue was also noted in the tunica intimae of the rabbits in the control group.

Pathologic morphology

The disrupted aortic wall structures were detected in HBO exposed rabbits microscopically when compared with control subjects. The thickening and foam cells were detected on tunica intimae and prominent vasovarums were observed in the study group with the hematoxylin-eosin staining of aortic tissue. PAS staining revealed both the undulation of the endothelium and localized separations of the tunica media layer. Moreover, similar findings were detected with the Masson's trichrome staining (**Figure 1**).

Discussion

Our results indicated that continuous HBO treatment has harmful effects on healthy aortic wall. We found disruptions in three

layers as endothelial undulation, thickening and foam cells on tunica intimae, and separations on tunica media in HBO treated rabbits when compared with normal genus. Moreover, prominent vasa vasorums were observed in study group. According to our knowledge, this is one of the few histomorphological studies that investigate the effects of continuous HBO exposure on the healthy aorta.

HBO treatment aims to high oxygen supply to ischemic cells and improves regenerative mechanisms. However, HBO induced reactive oxygen species on normally oxygenated tissue and negatively affects normal tissue cycle that results in micro and macro damage in an organism. Even harmful effects of HBO on DNA were reported in previous reports and the antioxidant supplies tried to prevent DNA damage due to continuous HBO exposure.[14,15] The main usage field of HBO is tissue healing via targeting proliferative effects of HBO. According to the report by Kang et al.[16] the continuous HBO treatment ameliorates fibroblast growth and directly affects autocrine growth factors. They found that HBO exposure leads to initial inhibition of fibroblast growth, but revelry effects after continuous treatment. These results might be related to a delayed cellular adaptation to high dose oxygen supply.[17,18] Previous reports suggested that HBO has secondary physiologic effects on inflammatory reaction by modulation of inflammation and immune function.[17-19] Gao et al.[20] concluded that HBO preconditioning modulates tissue healing by the stimulation of endogenous antioxidant and anti-inflammation defense systems and they indicated as HBO reduced inflammation via inhibiting pro-inflammatory cytokine production by monocyte-macrophages. Huang et al.[21] investigated the effects of HBO in an endothelin-1 induced cerebral ischemia model that can be related to endothelial cell damage or cerebral vasoconstriction. They found reduced infarct size with HBO treatment and they observed an enhanced ratio of Bcl-2 and Bax and a reduced expression of hypoxia-inducible factor-1α in animal models with focal cerebral ischemia after HBO application.[21] Unfirer et al.[22] hypothesized that HBO therapy might have benefits to the vascular system by modulating vessel contraction or dilatation in resistance vessels by regulating vascular activity and they described as this mechanism can possibly regulate the sensitivity of vessels in response to physiological stimuli of vasoconstrictor and vasodilator metabolites of arachidonic acid and nitric oxide. However, this hypothesis should be confirmed with animal studies and processes should be explained at the molecular level, because HBO also increases partial pressure of oxygen that can induce free oxygen radical release and cause harmful oxidative stress.[22] Furthermore, the systemic effect of HBO is still controversial, but a common claim confirms that HBO exposure leads to an increment in peripheral vascular resistance and HBO seems to compensate ischemic conditions at microcirculatory level.[23,24] In an *in vitro* animal aortic vessel preparation with a Krebs Ringer study, the effects of normobaric oxygen and HBO exposure were investigated in normal physiological and wound environments.[25] Any significant structural changes were not described in this *in vitro* study in regards to aortic intimae, media and adventitia layers and HBO therapy did not cause main differences in vascular endothelial growth factor, nitrite and nitrate levels in physiological conditions, but the investigators claimed that these factors significantly altered and vessel tissue become more responsive to HBO when wound conditions are mimicked.[25] It has been said that acute HBO may be beneficial in the development of vascular functions after revascularization, but the effect of repeated exposure has not been demonstrated.[26] Many studies have been conducted on the effects of oxidative stress on vascular aging. In the case of ongoing oxidative stress, all layers of the aorta are affected and structural disorders increase as the process lengthens.[27,28] It has been stated that nitric oxide-mediated endothelial response is affected and contractile response varies, especially in oxidative stress exposure.[28] It has been determined that repetitive stress causes deterioration in vessel wall response and continuous exposure negatively affects remodeling mechanisms.[29] The aorta is the main vessel coming out of the heart, and the blood distribution of the whole body is through this vessel. Therefore, systemic stressors also mainly affect the aorta.[30] Mihaljević et al.[31] indicated that acute HBO exposure increases superoxide formation and intermittent exposure may be beneficial for vascular relaxation. However, the effects of chronic oxidative stress have not been studied and the effect of continued exposure on vessel morphology has not been studied. In our *in vivo* study we investigated structural changes of HBO in the healthy aorta and we found disruption for all layers of aorta after exposure of continuous aorta.

The main limitation of this study is the fact that the results obtained from animal experiments should be confirmed in human subjects, because these results are preliminary and should be verified with further experimental researches. The other issue is the mechanism of vascular injury caused by HBO that was not detailed in the present pilot study. Therefore, it should clarify the cellular basis of injury in further analyses.

To sum up, HBO treatment seems to have hazardous effects on the healthy aorta. Damages to tunica intimae, media and adventitia layers have been detected after continuous HBO treatments. This speculative situation might be associated with triggered oxidative stress by HBO exposure. However, the tissue oxidative stress should be evaluated and the molecular effects of HBO should be clarified with further studies to understand the underlying mechanisms and confirm our results.

Author contributions
Study design, data collection, manuscript writing and supervision: CY; data collection and analysis: OK and OA. All authors contributed to the article and approved the submitted version of this manuscript.
Conflicts of interest
There are no conflicts of interest

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